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Bioenergetics of the obligate intracellular parasite *Rickettsia prowazekii*

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Abstract

Mitochondria are thought to be derived from an ancestor of the α-proteobacteria and more specifically from the Rickettsiaceae. The bioenergetic repertoire of the obligate intracellular parasite *Rickettsia prowazekii* is consistent with its postulated role as the ancestor of the mitochondria. For example, the *R. prowazekii* genome contains genes encoding components of the tricarboxylic acid cycle as well as of the electron transport system, but lacks genes to support glycolysis. In addition, the *R. prowazekii* genome contains multiple genes coding for adenine nucleotide translocators which enables this intracellular parasite to exploit the cytoplasmic ATP of its host cell as a source of energy. The aim of this review is to describe the different aspects of the bioenergetic system in *R. prowazekii* and to discuss the results of phylogenetic reconstructions based on a variety of bioenergetic molecules which shed light on the origin and evolution of the mitochondrial genomes. © 1998 Elsevier Science B.V.

Keywords: α-Proteobacterium; ATP/ADP translocase; Electron transport; Mitochondrion; (*Rickettsia prowazekii*)

1. Introduction

Rickettsia prowazekii belongs to the α-proteobacteria and is thought to be the closest modern relative of mitochondria [1–4] Fig. 1. Members of the genus *Rickettsia* grow and divide primarily within the eukaryote cell cytoplasm but some species are also capable of growth in the cell nucleus. *R. prowazekii* is an obligate intracellular parasite that grows exclusively in the host cell cytoplasm with a doubling time of around 10 h [5]. The eukaryotic cytoplasm differs from any other growth environment in that it provides a source of unexploited food and effectively eliminates interspecies competition. For the last half century, biochemists and microbiologists have been committed to understanding the fascinating biology of this organism [5,6]. However, experimental studies have been severely hampered by the intracellular nature of this organism and the difficulty to cultivate it outside of its eukaryotic host. Furthermore, no standard genetic tools such as transformation, transduction and/or conjugation systems are available. A complete genome-sequencing project has now provided new insights into the biology and evolution of *R. prowazekii*.

Members of the *Rickettsia* are distributed worldwide and normally established in arthropod hosts such as ticks, mites and insects, but they are also capable of infecting man and other vertebrates [7]. The genus *Rickettsia* comprise three main groups, the typhus group rickettsia (TG), the spotted fever group rickettsia (SFG) and the scrub typhus group rickettsia (STG). The typhus group consists of two species, *R. prowazekii* and *Rickettsia typhi*, the causative agents

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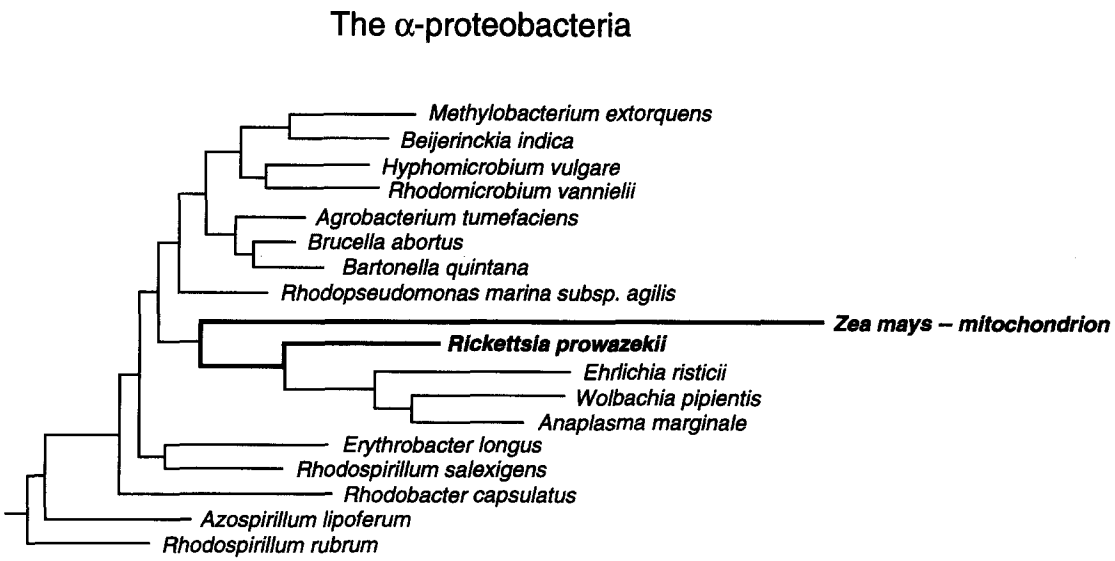

Fig. 1. Phylogenetic tree emphasizing the origin of mitochondria from within the group of α-proteobacteria to which the genus *Rickettsia* belongs. The tree is drawn essentially according to Ref. [1]; bold traces indicate the mitochondrial and Rickettsial lineages.

of epidemic and murine typhus, respectively. Members of the SFG also include species that cause disease in humans, such as for example *Rickettsia rickettsii* and *Rickettsia akari*, the causative agents of Rocky Mountain spotted fever and Rickettsial pox, respectively. The STG is composed of several different strains of a single species, which was previously referred to as *Rickettsia tsutsugamushi* but has recently been renamed as *Orientia tsutsugamushi* [8].

Adaptations to intracellular niches have occurred at numerous times in a variety of bacterial lineages independently of each other [9]. Comparative analyses of obligate intracellular parasites of different phylogenetic affiliation have provided nice examples of convergent evolution in response to the transition to the intracellular growth habitat. One such derived feature is a significantly reduced fraction of genes encoding components of the biosynthetic pathways in the obligate intracellular parasites *R. prowazekii* and *Chlamydia trachomatis* [10,11]. This may not be surprising; the energy and reducing power required for the biosynthesis of essential building blocks impose a heavy burden on the cell, and intermediates of most biosynthetic pathways are accessible in the host cell cytoplasm. Another shared feature of bacteria that multiply intracellularly is single copies of genes that are normally present in multiple copies per genome, such as the rRNA genes and the gene coding for elongation factor Tu [12–14]. Such a loss of otherwise essential genes partially explains the unusually small size (1 Mb) and the highly rearranged architectures of the rickettsial and chlamydial genomes [15,16].

On the basis of 16S rRNA and heatshock protein gene phylogeny, it has been proposed that mitochondria share a particularly close evolutionary relationship with members of the Rickettsiaceae [1–4]. Indeed, many genes encoding components that are characteristic of mitochondria have been identified in the *R. prowazekii* genome [11]. These include genes coding for enzymes of the tricarboxylic acid cycle and the respiratory chain complexes [11]. In addition, *R. prowazekii* possesses a unique nucleotide transport system for importing ATP directly from the host cell cytoplasm [17,18]. These systems will be discussed here, and compared to the bioenergetic systems of mitochondria. Special focus will be placed on the evolutionary relationships of the enzymes of the respiratory chain complexes and the ATP/ADP translocators in *Rickettsia* and mitochondria.

2. Citric acid cycle components

The rickettsiae are energy parasites (see below) but they can also generate their own metabolic energy by coupling the phosphorylation of ADP to ATP with the oxidation of glutamate via the tricarboxylic acid

cycle [6,19–22]. It has been shown that glucose is not catabolized by either intact cells [23] or extracts [24]. Indeed, genome sequence information suggests that *R. prowazekii* lacks key enzymes such as fructose-6-phosphate kinase required to support glycolysis (S.G.E. Andersson, unpublished data).

It is probable that pyruvate can be taken up from the cytoplasm, but this has not yet been demonstrated. Genes coding for enzymes of the pyruvate dehydrogenase complex (dihydrolipoamide dehydrogenase, dihydrolipoamide acetyltransferase and pyruvate dehydrogenase) which catalyze the oxidative decarboxylation of pyruvate to acetyl-CoA have been identified in the *R. prowazekii* genome (S.G.E. Andersson, unpublished data).

Enzymes of the tricarboxylic acid cycle have been partially purified [25]. Genome sequence information provides further evidence to suggest that *R. prowazekii* contains a functional tricarboxylic acid cycle. For example, genes coding for citrate synthase, aconitase, isocitrate dehydrogenase, succinyl-CoA synthase, succinate dehydrogenase, fumarase and malate dehydrogenase have been identified in the *R. prowazekii* genome ([11], S.G.E. Andersson, unpublished data). The gene encoding citrate synthase has been expressed in *Escherichia coli* and its regulatory control mechanisms have been studied [26]. There are two main types of citrate synthase: a 'small' dimeric enzyme composed of two identical subunits (mol. weight, ca. 100 000) that is primarily found in eukaryotic cells and gram-positive bacteria and a 'large' multimeric enzyme composed of four to six identical subunits (mol. weight, ca. 250 000) that has been found exclusively in gram-negative bacteria [27–29]. The citrate synthase in *R. prowazekii* is of the small type and is strongly inhibited by ATP but not by α-ketoglutarate or NADH [26,30].

3. Redox and electron transport components

The *R. prowazekii* genome has been found to contain many genes coding for enzymes of the electron transport system [11]. Rickettsial genes coding for components of the first of the three energy-coupling sites, the NADH dehydrogenase complex, are most similar to their homologs in α-proteobacteria and mitochondria (T. Sicheritz and S.G.E. Andersson, unpublished data). In the *Escherichia coli* genome, a total of nine genes coding for components of the NADH dehydrogenase complex are located in immediate proximity to each other (*nuoEFGHIJLKMN*). Part of this arrangement is also observed in the *R. prowazekii* genome, although in this species, the genes have split up into four different segments, with the gene clusters *nouGHI* and *nuoJKLM* being inversely oriented relative to each other and separated by the gene *ccmA* which codes for a putative heme exporter protein (S.G.E. Andersson, unpublished data). Two additional genes, *nuoF* and *nuoN*, are located far away from each other as well as from the *nuoGHI* and the *nuoJKLM* gene clusters in *R. prowazekii*.

The second coupling site of the respiratory chain system is the cytochrome bc_1 complex, which contains three proteins: cytochrome *b*, cytochrome *c*1 and the Rieske iron–sulfur protein. This complex has been isolated from a number of α-proteobacteria such as *Paracoccus denitrificans*, *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Rhodospirillum rubrum* and *Bradyrhizobium japonicum* [31]. The genes encoding the Rieske iron–sulfur protein (*fbcF*), cytochrome *b* (*fbcB*) and cytochrome *c*1 (*fbcC*) are clustered in *R. prowazekii* (T. Sicheritz and S.G.E. Andersson, unpublished data) as in *R. capsulatus*, *R. sphaeroides* and *P. dentrificans* [31]. *B. japonicum* is unusual in that two of these genes (*fbcB* and *fbcC*) have merged into one single gene (*fbhH*) [32].

The cytochrome oxidase complex, the third coupling site, catalyzes the transfer of electrons from ferrocytochrome *c* to molecular oxygen. Genes coding for subunits I and III of this complex, which in eukaryotes are normally encoded by the mitochondrial genomes have been identified in the *R. prowazekii* genome [33]. A gene coding for cytochrome *c*, has also been identified in the *R. prowazekii* genome (S.G.E. Andersson, unpublished data).

Finally, we have identified several genes involved in the biosynthesis of ubiquinone (*ubiA*, *ubiE* and *ubiX*), as well as genes involved in the biosynthesis of heme (*hemA*, *hemB*, *hemC*, *hemE*, *hemF*, *hemH*, *hemK* and *hemN*). Taken together, the information obtained from the sequence of the *R. prowazekii* genome strongly suggests that all of the essential

components required for an efficient electron transport system are present in *R. prowazekii*.

4. The ATP synthase gene operon

The ATP synthase gene operon (*atpIBEFHAGDC*) represents one the most highly conserved gene operon structures in bacterial genomes [34]. For example, this operon is identically organized in γ-proteobacteria such as *Escherichia coli* and *Haemophilus influenzae*, as well as in more distantly related Gram-positive bacteria such as *Bacillus subtilis* and *Mycoplasma genitalium*. The ATP synthase genes in *R. prowazekii* are organized into two gene clusters that are located distantly from each other (*atpBEF* and *atpHAGDC*) (S.G.E. Andersson, unpublished data). This observation supports the idea that the small *R. prowazekii* genome has been extensively rearranged and that many ancient genomic motifs have been disrupted during its evolution from a free-living bacterium to an obligate intracellular parasite [12–14].

5. The ATP/ADP translocase gene family

In addition to the oxidative phosphorylation of ADP to ATP, *R. prowazekii* can obtain energy by the exchange of rickettsial ADP for host cell ATP (Fig. 2). The exchange of ADP and ATP between the mitochondrial matrix and cytosol in eukaryotic cells is catalyzed by the adenine nucleotide translocator (ANT), which is present in the inner mitochondrial membrane. Extensive studies have been performed on the structure and transport functions of the mitochondrial ATP/ADP translocases [35–37].

For obligate intracellular parasites that grow directly in the ATP-rich cytoplasm it is beneficial to be able to use the ATP of their host cells. Indeed, efficient systems for nucleotide transport have been discovered in *R. prowazekii* as well as in *C. trachomatis* [17,38] but not in any free-living bacterial species. This suggests that the utilization of the host cytoplasmic ATP with the help of ATP/ADP translocases is an adaptation to the intracellular growth habitat. The occurrence of these transporters in intracellular parasites of distant phylogenetic relationships provides a nice example of convergent evolution.

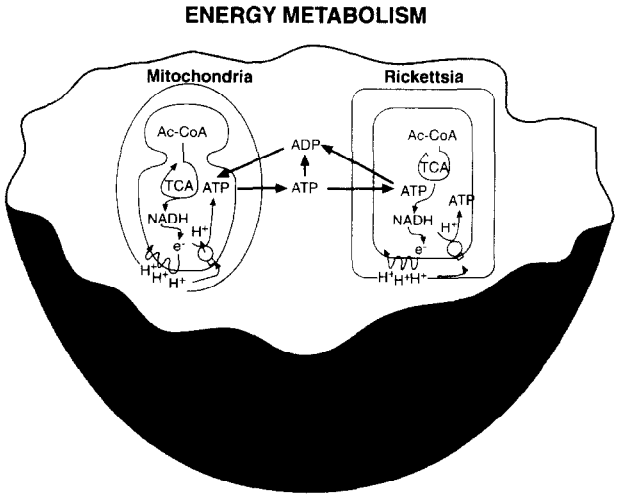

Fig. 2. Schematic picture showing the exchange of ATP and ADP between the eukaryotic host cell cytoplasm, mitochondria and *Rickettsia*. The presence of citric acid cycle components, electron transport components and ATP synthase in mitochondria and *Rickettsia* is schematically shown.

The membrane-bound nucleotide translocase in *R. prowazekii* is specific for ATP and ADP [17]. During an early phase of the infection the concentration of ATP is higher in the host cell cytoplasm than inside *R. prowazekii*, and the net direction of ATP transport is therefore from the host cell cytosol into the cytoplasm of the parasite. However, the metabolic capacity of the host cell gradually becomes more and more compromised and during later stages of the infectious cycle *R. prowazekii* starts producing its own ATP by oxidative phosphorylation. When the concentration of ATP inside the parasite has increased significantly relative to that of the host cell cytoplasm, continued exchange of nucleotides would be disadvantageous for the parasite since it would lead to the sequestering of ATP into the host cell cytoplasm. It has been shown that the expression of the ATP/ADP translocase gene is downregulated in heavily infected host cells [39]. By contrast, it has been shown that the expression level of *gltA*, which codes for citrate synthase, is most highly expressed during later phases of the infection [39].

The gene encoding the *R. prowazekii* ATP/ADP translocase (*tlc*) has been expressed in *E. coli* [40] and the translocase has been solubilized and actively reconstituted into proteoliposomes [41]. With the help

of antibodies the carboxyl terminus of the ATP/ADP translocase has been localized to the cytoplasmic side of the bacterial inner membrane [42]. An initial topological model has predicted 12 hydrophobic α-helical transmembrane regions and a putative disulfide bridge between Cys^{37} and Cys^{85} [42]. However, the disulfide bond appears not to be functionally required since the transport properties of the translocase were observed to be unaffected by site-directed mutagenesis of the cysteine residues to alanines [43].

Genetic analyses have shown the presence of a multitude of isoforms of the mitochondrial ADP/ATP translocators in a variety of eukaryotes [44–58]. We have putatively designated five genes as coding for ATP/ADP translocases in the *R. prowazekii* genome (R. Podowski and S.G.E. Andersson, unpublished data). The amount of mRNA transcribed from four of the homologs were found to be comparable, suggesting that all members of this family represent genes that are actively transcribed and translated (H.H. Winkler and S.G.E. Andersson, unpublished data). Phylogenetic reconstructions have shown that the five ATP/ADP translocase genes duplicated and diverged from each other prior to the divergence of the typhus and the spotted fever group *Rickettsia* (R. Podowski and S.G.E. Andersson, unpublished data).

6. Evolutionary aspects of the bioenergetic machinery

The acquisition of mitochondria represents one of the most important events in the early history of the eukaryotes. It is generally believed that the ancestral endosymbiont supported an early version of the eukaryotic cell with energy in the form of ATP. Genes encoding components of the respiratory systems are therefore expected to provide particularly important information to questions concerning the origin and evolution of mitochondria. For such an analysis it is necessary that the closest modern bacterial relatives of mitochondria are compared to the most highly conserved mitochondrial genomes.

Recently, the sequence of the mitochondrial genome of the fresh-water protozoon *Reclinomonas americana* was determined [59]. This species has been classified with the jakobids, which share characteristics with the amitochondriate retortamonads, and is thought to have diverged from the main eukaryotic lineage prior to the acquisition of mitochondria [60,61]. The mitochondrial genome of *R. americana* is more bacteria-like than any other mitochondrial genome sequenced so far with respect to the number of genes it contains, as well as to the way in which these genes are organized. Approximately half of the genome codes for proteins involved in information processes, whereas the other half codes for bioenergetic enzymes [59]. Because of its deeply diverging position in the eukaryotic tree, the mitochondrial genome of *R. americana* is particularly well suited for phylogenetic analyses focusing on the origin and evolution of the bioenergetic components in mitochondria.

Phylogenetic reconstructions based on the ribosomal protein genes L2, L10 and L11 and the genes *rpoBC* which code for RNA polymerase β and β′ genes provide strong support for a close phylogenetic affiliation between *R. prowazekii* and the mitochondrial genome of *R. americana* (S. Jossan and S.G.E. Andersson, unpublished data). However, for many ribosomal protein genes the level of sequence conservation is too low to accurately resolve the details of the relationship between mitochondria and bacteria. Since all of the mitochondrial ribosomal protein genes in *R. americana* are located within genomic contexts that are characteristic of bacterial genomes (i.e., the *rif*, *str*, S10, *spc* and α operons), the entire mitochondrial information system must have been derived from one and the same endosymbiotic event [59].

Phylogenetic reconstructions based on concatenated sequences of cytochrome *c* oxidase subunit 1 and cytochrome *b* of the bc complex have shown that the mitochondrial genomes can be traced back into the α-proteobacteria, with some support for a particularly close phylogenetic relationship between mitochondria and *R. prowazekii* [35]. Phylogenetic reconstructions based on subunits of the ATP synthase complex also support an origin of the mitochondria from within the α-proteobacteria (T. Sicheritz and S.G.E. Andersson, unpublished data). Based on the sequences of cytochrome *b* and cytochrome *c* oxidase subunit 1, we have estimated that mitochondria and members of the Rickettsiaceae diverged 1500–2000 million years ago [35], which is in very

good agreement with the suggested time-span for the origin of mitochondria based on geochemical reasonings [62].

In contrast to components of the ATP synthase and the respiratory chain complexes, the rickettsial and mitochondrial ATP/ADP translocases appear not to be evolutionarily related to each other. For example, the rickettsial translocases are monomers with 12 transmembrane segments, whereas the mitochondrial translocases consist of dimers with six transmembrane segments. Furthermore, no alignable regions could be identified for the mitochondrial and rickettsial types of ATP/ADP translocases (R. Podowski and S.G.E. Andersson, unpublished data). This suggests that efficient systems for ATP and ADP transport arose independently of each other in mitochondria and *Rickettsia* (R. Podowski and S.G.E. Andersson, unpublished data).

Nuclear genes coding for plastid ATP/ADP translocases have recently been found in the plants *Arabidopsis thaliana* and *Solanum tuberculosum* [63]. Surprisingly, the plastid ATP/ADP translocases were observed to display strong sequence similarities to the bacterial ones but not to the mitochondrial type of translocases ([63]; R. Podowski and S.G.E. Andersson, unpublished data). The evolutionary relationship of the bacterial and plastid nucleotide transport systems remains to be explained.

7. Concluding remarks and open questions

Phylogenetic reconstructions based on numerous genes encoding components of the information and the bioenergetic systems of *R. prowazekii* suggest that mitochondria have originated from the α-proteobacteria and share a common ancestor with members of the Rickettsiaceae. However, in contrast to the close phylogenetic affiliation of the mitochondrial respiratory chain proteins with those from the α-proteobacteria, the origin and evolution of the nucleotide transport systems in *R. prowazekii* and mitochondria remains elusive. The data currently available suggests that there are at least two types of ATP/ADP translocases that have originated and evolved independently of each other in bacteria and mitochondria. This raises intriguing questions about the enzymatic capabilities of their common ancestor and the nature of its relationship with the eukaryotic cell.

While it can be assumed that the ancestral endosymbiont was capable of oxidative phosphorylation and ATP production, it is unclear whether efficient mechanisms for nucleotide exchange were established prior to the endosymbiotic event. On the one hand, it is possible that the mitochondrial ATP/ADP translocase genes evolved subsequent to the transformation of the original endosymbiont into an organelle. Alternatively, two paralogous gene families of ATP/ADP translocases may have been transferred from the endosymbiont into the eukaryotic cell, one of which was retained by the mitochondria and the other subsequently recruited by the plastids. Independent of the precise mechanism by which a group of α-proteobacteria evolved into energy-generating systems of the eukaryotic cell, one of their sister lineages, the *Rickettsia,* escaped endosymbioses and later specialized into obligate intracellular parasites that learnt how to exploit the mitochondrially generated ATP as a source of energy.

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